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ARTICLE TYPE

Oxidation of thiols to disulfides by dioxygen catalyzed by a bioinspired organocatalyst†

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2,3-Dihydro-2,2,2-triphenylphenanthro[9,10-*d*]-1,3,2-□^{5}-oxazaphosphole serves as good catalyst for the oxidation of thiophenol, cysteine and glutathione to their disulfides by molecular oxygen. The kinetics of the reactions unveiled an overall second order rate equation for all reactions and pure dioxygen chemistry for all three substrates. The formation of an unstable hydroperoxide from the catalyst is assumed as a key step during the reaction.

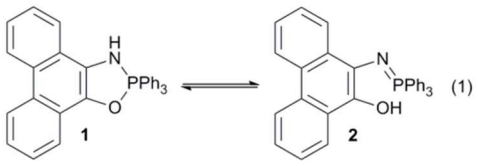
Oxidative coupling of thiols to disulfides has attracted interest in biological[1] and synthetic contexts.[2] Cysteine residues are possible catalysts for protein modification[3] and quiescinsulfhydryl oxidases[4] are responsible for oxidative protein folding. Glutathione[5] and disulfides[6] prevent oxidative stress and oxidative damage in biological systems.

Oxidative coupling of thiols is a very common reaction for the synthesis of disulfides. Oxidative S-S coupling can be achieved by permanganate,[7] halogens,[8] $FeCl_3$,[9] organometallic complexes,[10] peroxides[11] and nanoparticles.[12]

In this paper we report that an 1,3,2-oxazaphosphole is a good bioinspired catalyst for the oxidation of thiophenol, cysteine and glutathione to their corresponding disulfides by triplet dioxygen.

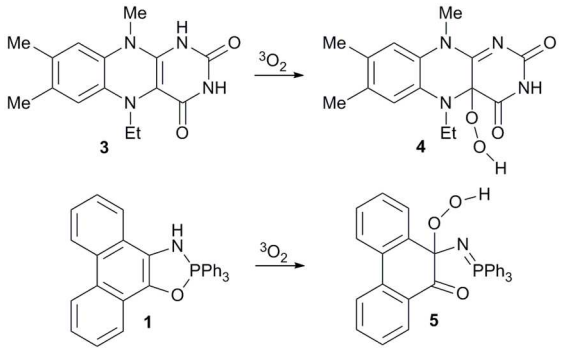
1,2-Quinones undergo with tertiary phosphines a [4+1] electrocyclic reaction giving dioxaphosphole heterocyclic rings.[13] 1,2-Azaquinones give 1,3,2-oxazaphospholes (**1**) by the use of tertiary phosphines in good yields.[14,15] Where the monoimines are unstable they can also be prepared „in situ“ from the corresponding quinones and triphenylphosphine in a sealed tube under ammonia. Electron-releasing groups on the quinones make the nucleophilic replacement of the C=O bond with NH_3 to a C=NH functionality difficult or impossible (*e. g.* NMe_2, OR).

1,3,2-Oxazaphospholes exhibit tautomerism as shown in Scheme 1 between the oxazaphosphole form (**1**) and the iminophosphorane form (**2**) dependent on the consistency or the solvent of solution (Scheme 1).[14,15] 1,3,2-Oxazaphospholes (**1**) seem to be stable in the solid form. In solution, however the tautomeric compounds of the iminophosphorane form (**2**) are also present. ^{31}P NMR spectra in the range of 39-51 ppm are characteristic for **1** and those of the form **2** have a shift in the range from -23 to -8 ppm. In more basic solvents the oxazaphosphole form **1** is dominant. In solution they decompose readily in air due to dioxygen or moisture.



Scheme 1 The tautomeric forms of 1,3,2-oxazaphosphole (**1**).

1,3,2-Oxazaphospholes mimic flavoproteins,[16] pterins[17] and deprotonated uric acid[18] (*e. g.* Scheme 2). That means that they react with triplet dioxygen to form unstable



Scheme 2 Similarities of flavoprotein and 1,3,2-oxazaphosphole reactions with $^3O_2$.

hydroperoxide, as shown earlier.[19] These hydroperoxides are then able to oxidize/oxygenate various substrates, such as PPh_3[19] and CO_2.[20] We extended the oxidation by $^3O_2$ catalyzed by the 1,3,2-oxazaphosphole **1** to thiols, such as thiophenol, cysteine and glutathione. Thiophenol represents thiols, while cysteine oxidation to disulfide has some relevance to protein folding[3,4] and glutathione is very important against oxidative stress in biological systems.[5,6] Bulk oxidation of the substrates in methanol or in methanol/water mixture at 298 K resulted in yields of 93, 92 and 76% (GC and O_2-uptake, isolated yields:

78, 70 and 65%) of the disulfide. In the first cases the yields were calculated from the GC and dioxygen-uptake (see ESI general part and Fig. S1). No other oxidized sulfur-containing products were formed, though they have been detected in numerous other oxidation formats (H_2O_2 and peroxy acids, for example, may lead to sulfinic acid.[21,22] We measured autoxidation of the substrates under identical condition and deduced their rate from the catalytic reactions (Fig. S2 - Fig S4). The disulfides were characterized by comparing them with authentic samples (mps, mixed mps, IR spectra, MS and NMR). GC-MS (thiophenol), ^{1}H- and ^{13}C-NMR (cysteine and glutathione)) (Fig S.5 - Fig. S12). Kinetic studies on the oxidation of the three substrates were carried out measuring the disulfide amount by gas chromatograph (thiophenol) and uptake of $^3O_2$ in the case of cysteine and glutathione (Fig. S1). Typical time courses can be seen in Fig. 1. The oxidation reactions have been carried out in a thermostated vessel connected to a gas buret filled with O_2 (constant pressure) and a septum to take samples for GC. Duplicate runs were done in each case.

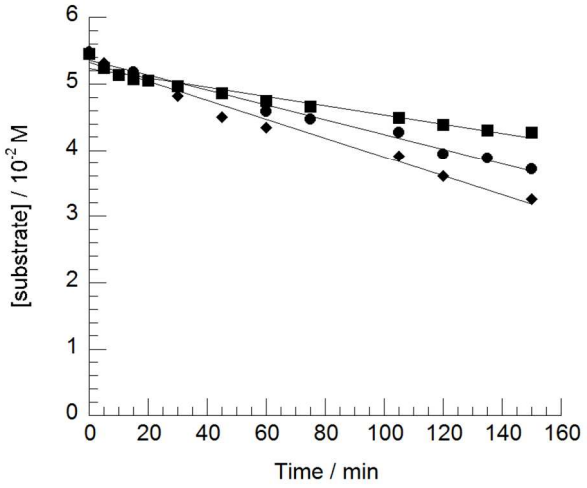


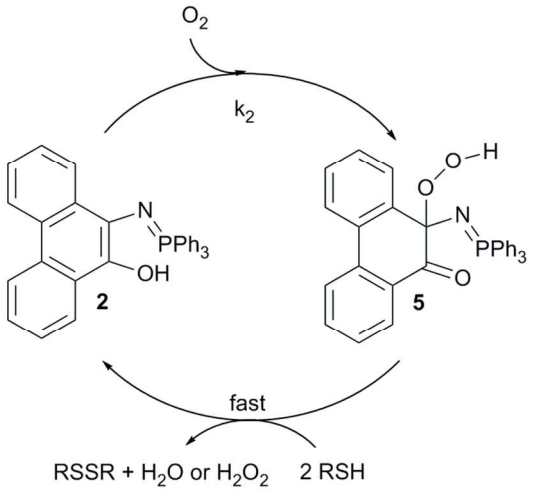
Figure 1 Time course of the oxidation of thiols by $^3O_2$ catalyzed by **1**.
● [cysteine] = 5.50×10^{-2} M, [1,3,2-oxazaphosphole] = 6.00×10^{-4} M, [O_2] = 3.54×10^{-3} M, V(MeOH:H_2O=2:1) = 30 mL, T = 25 °C.
■ [glutathione] = 5.50×10^{-2} M, [1,3,2-oxazaphosphole] = 6.00×10^{-4} M, [O_2] = 3.54×10^{-3} M, V(MeOH:H_2O=2:1) = 30 mL, T = 25 °C.
♦ [PhSH] = 5.50×10^{-2} M, [1,3,2-oxazaphosphole] = 6.00×10^{-4} M, [O_2] = 9.50×10^{-3} M, V(MeOH) = 30 mL, T = 25 °C.

The partial rate orders of the reaction were secured also by plotting the initial concentrations of PhSH, cysteine and glutathione against the reaction rates (Fig. S13 – Fig. S15), which gave also straight lines. These Figures, where the reaction rates remained constant, reinforced the zero order dependence on the thiol concentrations. Plotting the reaction rates against the various starting concentrations of **1** and dioxygen resulted also in straight lines (Fig. S16 - Fig. S21) indicating first order dependence on **1** and dioxygen concentration. The kinetic data suggested an overall second order rate equation (1) and the second order rate constants are

$$\text{Reaction rate} = k_2\,[\text{catalyst}]\,[^3O_2] \qquad (1)$$

$k_2^{\text{PhSH}} = 5.5 \pm 0.3 \times 10^{-1}$ $M^{-1}s^{-1}$, $k_2^{\text{cysteine}} = 12.5 \pm 1.8 \times 10^{-1}$ $M^{-1}s^{-1}$ and $k_2^{\text{glutathione}} = 4.2 \pm 0.5 \times 10^{-1}$ $M^{-1}s^{-1}$ with the activation parameters PhSH: $\Delta E_a = 24.5 \pm 0.4$ kJmol^{-1}, $\Delta H^{\ddagger} = 21.6 \pm 1.1$ kJmol^{-1}and $\Delta S^{\ddagger} = -177.0 \pm 0.4$ Jmol^{-1}K^{-1}, cysteine: $\Delta E_a = 8.1 \pm 0.1$ kJmol^{-1},$\Delta H^{\ddagger} = 5.6 \pm 0.1$ kJmol^{-1}, $\Delta S^{\ddagger} = -224.2 \pm 0.2$ J mol^{-1} K^{-1} and glutathione: $\Delta E_a = 9.9 \pm 0.1$ kJmol^{-1}, $\Delta H^{\ddagger} = 7.0 \pm 0.1$ kJ mol^{-1}, $\Delta S^{\ddagger} = -228.7 \pm 0.2$ Jmol^{-1}K^{-1} (Fig. S22 - Fig. S27 and Tables S1 - S3).

This means that in these of reactions the catalyst reacts with triplet dioxygen in a slow step and this is rate-determining. The formation of the hydroperoxide **5** determines the scenario. All the consecutive reactions of the hydroperoxide **5** with thiophenol, cysteine and glutathione and the formation of the disulfides, are fast reactions, which do not influence the reaction rates. The reactions can be considered as pure dioxygen chemistry as depicted in Scheme 3. If this is true the reaction rates must be close to each other. Of course we have to consider that in the case of cysteine and glutathione MeOH:H_2O = 2:1 mixture have to be used as solvent due to solubility reason. This roughly holds if we look at the rate constants. However, the O_2-solubility in the solvents and the various substrates probably have some effect on the reaction rates too. There is also a difference in the product formation. In the case of PhSH



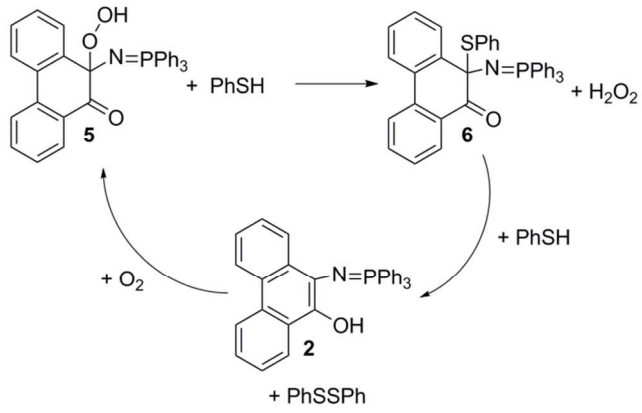
Scheme 3 The proposed mechanism of thiophenol, cysteine and glutathione oxidation by $^3O_2$ catalyzed by **2**.

hydrogen peroxide is formed (that was determined from dioxygen-uptake and also iodometric titration to be 87%),[23] while in the case of cysteine and glutathione water. Since all these steps are after the rate-determining step the kinetic data do not help us, we can only speculate or reasonable arguing what can be assumed.

Based on that we believe that the hydroperoxide **5** reacts in a fast reaction with the thiophenol, which is rather acidic, to an adduct (**6**) and H_2O_2, and a further reaction of thiophenol with **6** to the disulfide and the catalyst (Scheme 4). It is interesting to note that in the case of thiophenol oxidation beside of the disulfide hydrogen peroxide is formed, while in the case of cysteine and glutathione water. These reactions, after the rate-

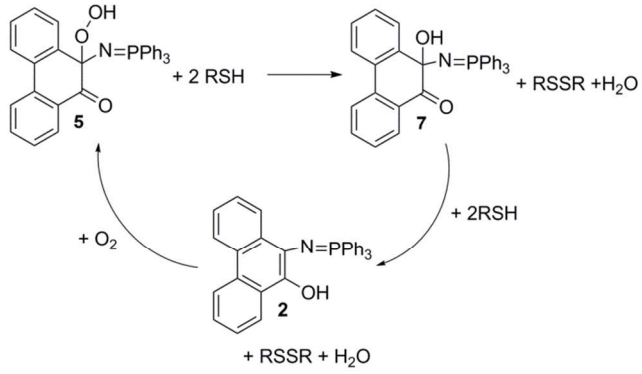
determining step, are fast ones and to our believe the unstable hydroperoxide (**5**) reacts with PhSH deliberating H_2O_2 and the compound **6** is formed. Compound like **6** was assumed in the reaction of an electron-deficient isoalloxazine, 3,10-dimethyl-8-cyano-isoalloxazine with thiophenol.[24] Compound **6** reacts then with a further thiophenol molecule to give the disulfide and the catalyst **2**. This reacts then again with O_2 resulting in **5** just to close the catalytic cycle.

If we look at the substrates cysteine or glutathione rather different pictures were found. In the case of cysteine and glutathione again a dioxygen chemistry could be observed, what means that the rate-determining step of the reaction is the reaction of the catalyst (**2**) with triplet molecular oxygen, which reacts in a fast consecutive reaction with the cysteine or glutathione and water and the catalyst is reformed.



Scheme 4 The proposed fast reactions of the hydroperoxide **5** with thiophenol.

All consecutive reactions after the rate-determining step are fast but are different in nature (Scheme 5). We believe that the reaction of the hydroperoxide **5** with cysteine or glutathione



Scheme 5 The **1**-catalyzed oxidation of cysteine and glutathione after the rate-determining step.

leads to similar OH-adducts as previously described, just providing both O-atoms for the formation of water. The first step is a typical hydroperoxide reaction,[25] and in the second step the **7** hydroxo compound provides the second O-atom with two thiol hydrogens for the formation of water. It resembles the classical hydroperoxide oxidation of the substrates.

Conclusions

In summary, we have disclosed that a 1,3,2-oxazaphosphole (**1**) catalyses the oxidation of thiophenol, cysteine and glutathione by molecular oxygen to their corresponding disulfides. The oxidation of thiols to disulfides has some importance on the structure of proteins, and in the last case glutathiones role as antioxidant in the cell is well recognized. Kinetics of the reactions resulted in an overall second order rate equation. That means that all the reactions can be called as pure dioxygen chemistry, since the rate-determining step is the reaction of the catalyst with molecular oxygen forming the hydroperoxide (**5**). After that **5** reacts with thiophenol to H_2O_2 and in the case of cysteine and glutathione to water and the corresponding disulfides. 1,3,2-Oxazaphospholes in these cases mimic flavoproteins exhibiting similar mechanistic features.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details of synthesis and structural characterization, kinetics and spectroscopic data. For ESI and electronic format see DOI: 10.1039/b000000x/

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